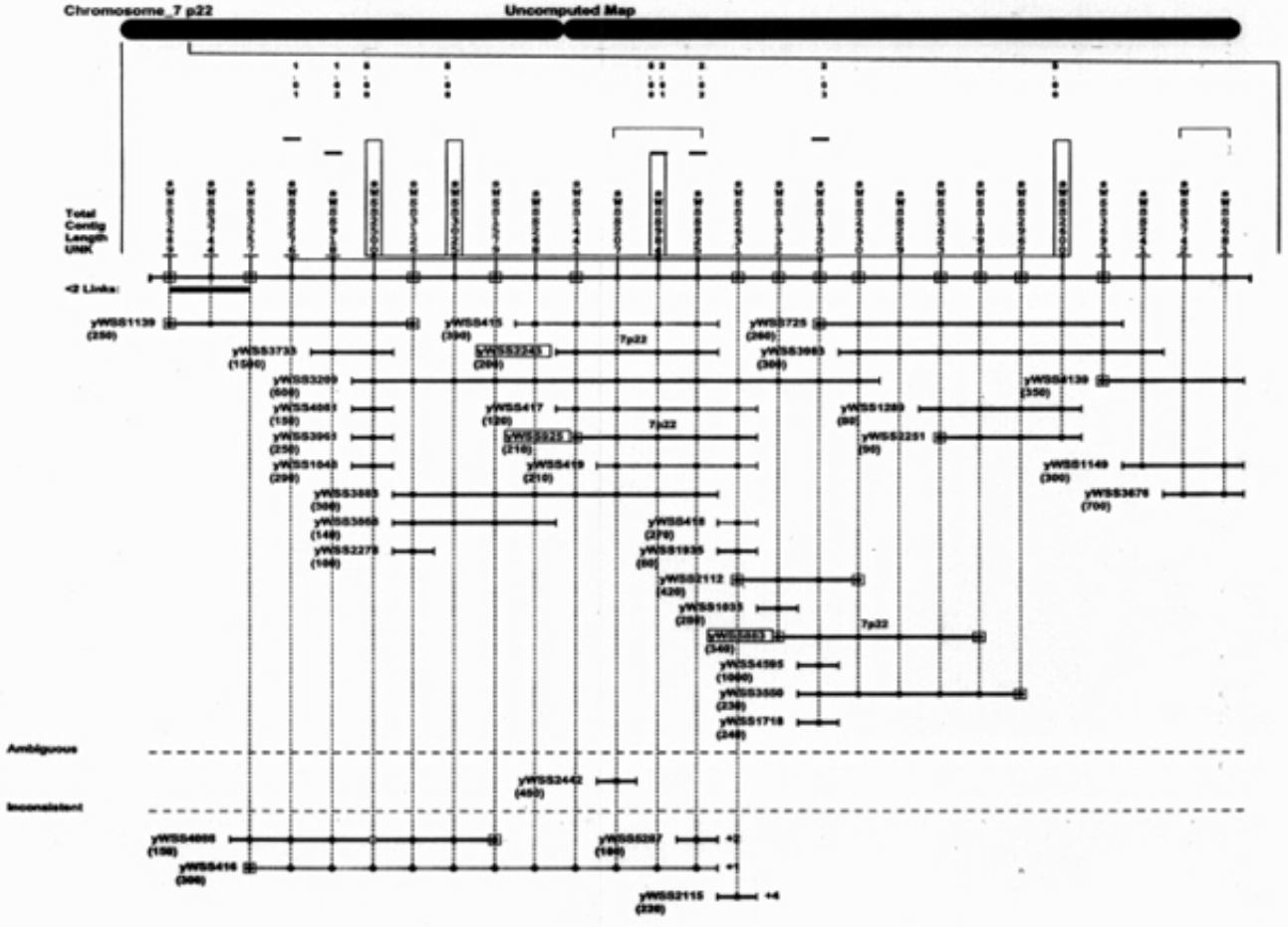
CLONE-BASED PHYSICAL MAPPING



Eric D. Green, M.D., Ph.D.
Chief, Genome Technology Branch (NHGRI)
Director, NIH Intramural Sequencing Center

Phone: 301-402-0201
FAX: 301-402-4735
E-Mail: egreen@nhgri.nih.gov

Clone-Based Physical Mapping

I. Fundamentals of Physical Mapping

II. Large DNA Cloning Systems

   A. Cosmids (Fosmids)
   B. P1s
   C. PACs
   D. BACs
   E. YACs

III. Strategies for Physical Mapping

IV. Clone-Based Physical Maps of Mammalian Genomes

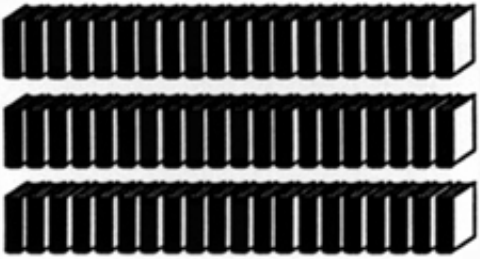
V. Future Prospects

General Plan for Lecture:

- Stress the Practical Aspects of Physical Mapping
- Focus on the Mapping of Mammalian Genomes
- Highlight Relevant Literature
- Provide Information on Relevant Electronic Resources

Genome Sizes

Human Genome



3,000,000,000 bp

Human Chromosome (average)

130,000,000 bp

Fruit Fly Genome

160,000,000 bp

Nematode Genome

100,000,000 bp

Yeast Genome

15,000,000 bp

E. coli Genome

5,000,000 bp

Fundamentals of Physical Mapping

- Importance of Physical Maps:

  Localization and Isolation of Genes (e.g., Positional Cloning)
  Study of Genome Organization and Evolution
  Framework for Systematic DNA Sequencing

- "Mapping is About Order"
  -Maynard Olson (1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999...)

- Physical Mapping Involves:

  Ordering of Clones and/or Landmarks
  Typically with Some Physically Measurable Metric

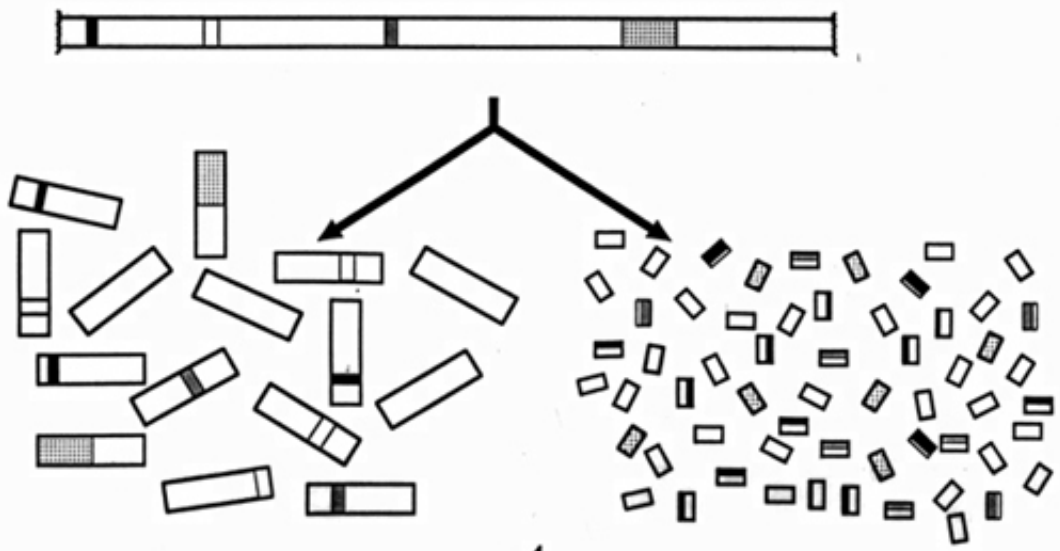
- Examples of 'Landmark Only' Ordering:

  Restriction Mapping by Pulsed-Field Gel Electrophoresis
  Radiation Hybrid (RH) Mapping

- Theoretical Discussions of Clone-Based Physical Mapping

  Arratia et al. (1991), Barillot et al. (1991),
  Palazzolo et al. (1991), Olson and Green (1993)

- Clone-Based Physical Mapping: 'Jigsaw Puzzle Analogy'



Large DNA Cloning Systems

- Reasonably Arbitrary Definition of 'Large'
- Bacterial- (Dunham et al. 1998) vs. Yeast-Based Host Systems
- Want the Cloned DNA to Accurately Reflect the Starting Genome

  Problem of Instability
  Problem of Chimerism

- Development of 'Array Mentality' for Clone Libraries

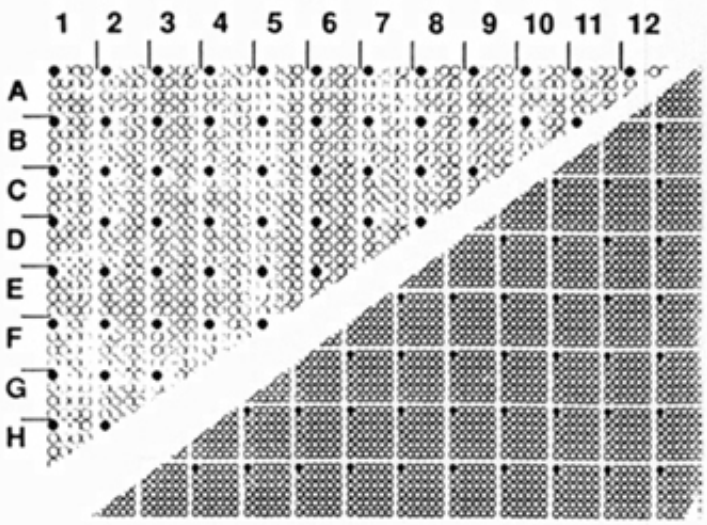
  Clones Arrayed in Individual Wells of Microtiter Plates
  Various Densities Available (96-, 192-, and 384-Well Plates)

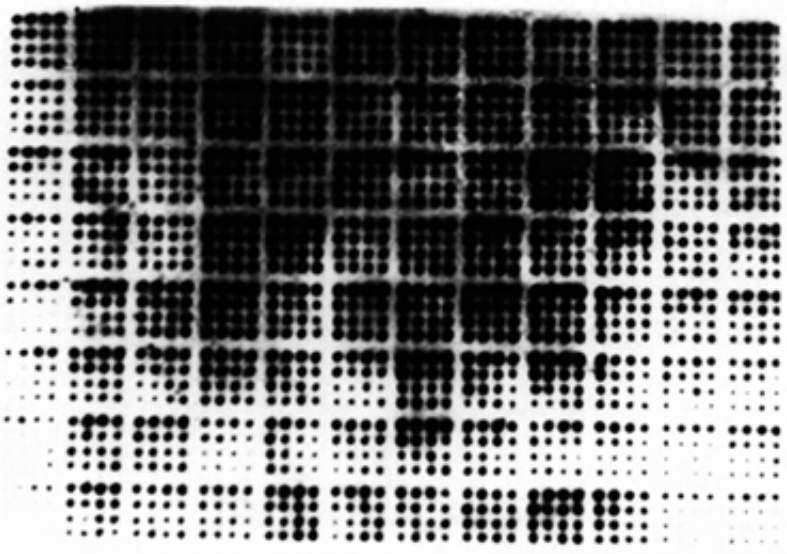
- Advantages of Arrayed Clone Resources ('Reference Libraries')

  1. Simplicity of Storing and Transferring Clone Collections
  2. Repeated Hybridization-Based Screening
  3. Repeated PCR-Based Screening
  4. Convenient Format for Retrieving Clones of Interest
  5. Ability to Assimilate Data on Common Clones

Hybridization-Based Analysis of High-Density Arrays

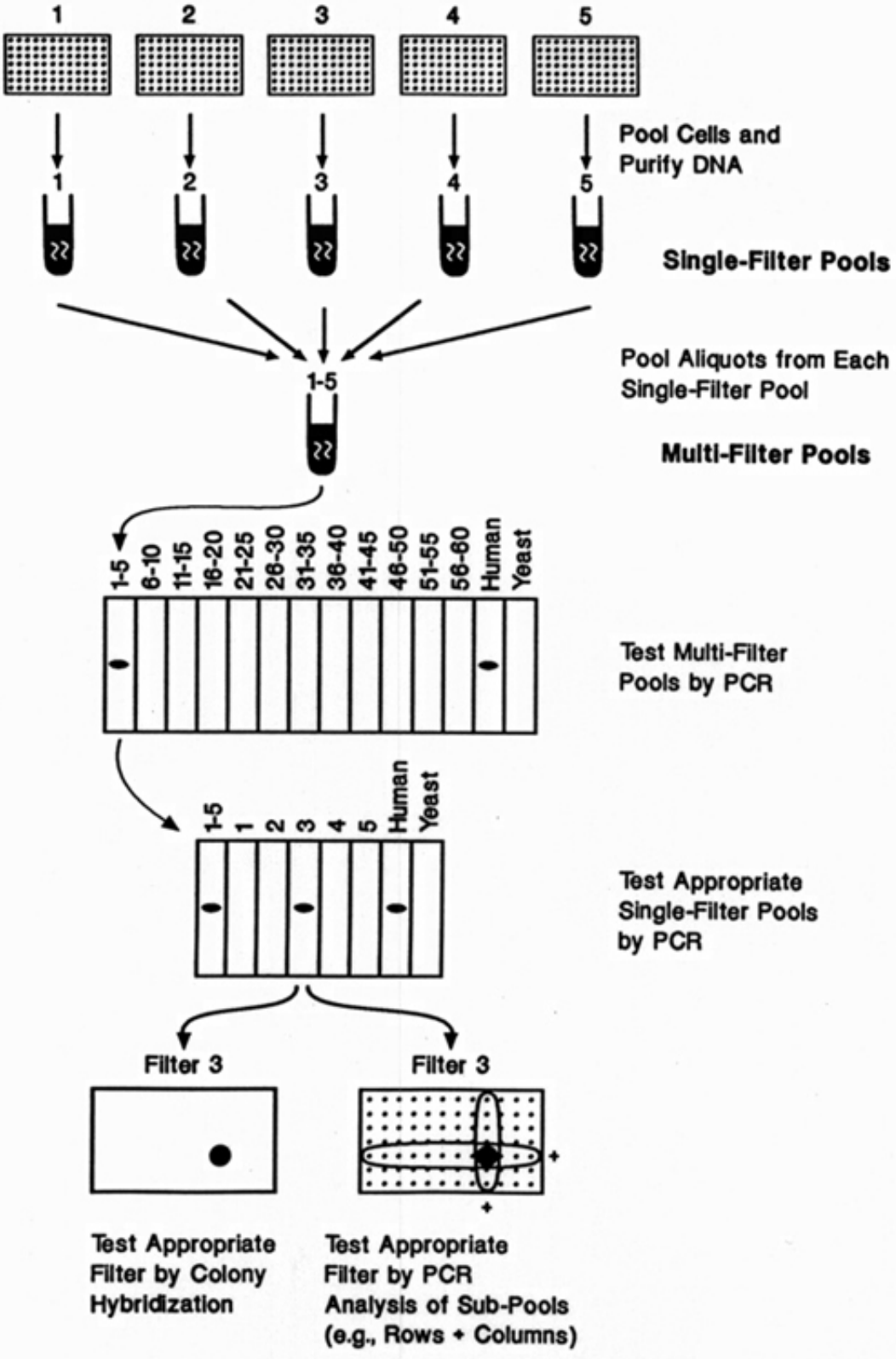
Bentley et al. (1992), Ross et al. (1992), Olsen et al. (1993)





PCR-Based Analysis of Arrayed Libraries

Green and Olson (1990)



Commercial Involvement in Clone Distribution

Research Genetics, Inc.

800-533-4363
205-533-4363
http://www.resgen.com

Genome Systems, Inc.

800-430-0030
314-692-0033
http://www.genomesystems.com

ATCC

800-638-6597
703-365-2700
http://www.atcc.org/

Cosmids

- Bacterial-Based Cloning System
- 'Antique' of the Large DNA Cloning Systems
- Plasmid Vector with Bacteriophage Packaging Sequences (*cos* Sites)
- High Efficiency Packaging System
  - Relatively Homogeneous Insert Sizes
  - Libraries from Small Amounts of DNA (e.g., Flow-Sorted DNA)
  - Antibiotic Selection
- Cloned Inserts: 35-45 kb, Circular DNA
- High Copy Number
  - High Yields of DNA by Standard Methods
  - Instability Problems (Despite Recombination-Deficient Hosts)
- Relatively Non-Chimeric
- Various Libraries (Whole Genomes, Individual Chromosomes)
- References
  - Sambrook et al. (1989), Wahl et al. (1987), Nizetic et al. (1991), Evans et al. (1992), Ivens et al. (1993), Evans (1998)
- 'Fosmids' [Kim et al. (1992)]
  - Cosmid Vector Engineered with F Factor
  - Low Copy → More Stable

P1 Clones

- Bacterial-Based Cloning System

- Developed by Sternberg (1990)

  Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs

  (DNA packaging/*pac* cleavage/genome mapping/gene isolation)

  NAT STERNBERG

- Bacteriophage P1 (Genome Size: 100 kb)

- P1-Based Vector and Complex P1 Packaging Extracts

  Limited to 100 kb (Constraints of Viral Particle)
  2 loxP Sites Results in Circularization of DNA
  Antibiotic Selection

- Cloned Inserts: 70-100 kb, Circular DNA

- Low Copy Number

  Low Yields of DNA by Standard Methods
  Highly Stable (with Recombination-Deficient Hosts)
  Potential for IPTG Induction→ 10-30 Fold Increase

- Relatively Non-Chimeric

- Human and Mouse Libraries Commercially Available

- References

  Sternberg (1990), Sternberg et al. (1990), Pierce and Sternberg (1992), Shepherd et al. (1994), Sternberg (1998)

P1-Derived Artificial Chromosomes (PACs)

- Bacterial-Based Cloning System

- Developed by Ioannou et al. (1994)

A new bacteriophage P1-derived vector for the propagation of large human DNA fragments

Panayiotis A. Ioannou[1], Chris T. Amemiya[1], Jeffrey Garnes[1], Peter M. Kroisel[1], Hiroaki Shizuya[2], Chira Chen[1,3], Mark A. Batzer[1] & Pieter J. de Jong[1,3]

- Slightly Modified P1 Vector
  - Lacks Packaging Signal
  - Antibiotic Selection

- Transform by Electroporation

- No Packaging of DNA → Larger Size Capacity

- Cloned Inserts: 100-150 kb, Circular DNA

- Low Copy Number
  - Low Yields of DNA by Standard Methods
  - Highly Stable (with Recombination-Deficient Hosts)

- Relatively Non-Chimeric

- Human and Mouse Libraries Available (see http://bacpac.med.buffalo.edu)

Bacterial Artificial Chromosomes (BACs)

- Bacterial-Based Cloning System
- Developed by Shizuya et al. (1992)

> **Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor-based vector**
>
> (electroporation/physical mapping/human genome)
>
> HIROAKI SHIZUYA*, BRUCE BIRREN, UNG-JIN KIM, VALERIA MANCINO, TATIANA SLEPAK, YOSHIAKI TACHIIRI, AND MELVIN SIMON†

- Based on the *E. coli* F Factor (Fertility Plasmid): Replication Control
- BAC Vectors
  - Cloning site in LacZ Gene (Blue/White Selection)
  - Antibiotic Selection
- Transform by Electroporation
- No Packaging of DNA → Larger Size Capacity
- Cloned Inserts: 100-200 kb, Circular DNA
- Low Copy Number
  - Low Yields of DNA by Standard Methods
  - Highly Stable (with Recombination-Deficient Hosts)
- Relatively Non-Chimeric
- Numerous Libraries Available (see http://bacpac.med.buffalo.edu)
- See Birren et al. (1998)